# بیولوژی ملکولی سلول
## دوره کارشناسی نا پیوسته علوم ازمایشگاهی
## دکتر ساروخانی

Dr . Sarookhani

# CELL BIOLOGY BRANCHES

Cytology

Cell physiology

Cell anatomy

Cytochemistry

Cytogenetic

Cell biophysics

Molecular biotechnology

Genetic engeneering

Molecular biology

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# Molecular Biology


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# CELL& MOLECULAR BIOLOGY APPLICATIONS

Gene therapy
Recombinant proteins
Chromosome & gene study
Transgenic plants
Transgenic animals
Cell culture & tissue engineering
Stem cell applications
IVF
PROTEIN ENGINEERING
OTHERS

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# THE CELL

- The smallest living component of biological system
- the smallest living level of organization
- the smallest reproducible unit of life that can be recognized as a basic elementary organism

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# GENERAL CHARACTERISTICS OF THE CELL

1)has a genome as a information molecule
2)its genetic information can mutate
3)has metabolism
4)has a cytoplasmic membrane
5)is irritable(responding to stimulation)
6)has motility

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- Cells differs greatly in size & shape

- Muscle cell 30 cm long ; RBC 7 µm diameter

- Building blocks of the body

- Shape is maintained by protein filaments ~ *cytoskeleton*

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# BIOLOGICAL ELEMENTS CLASSIFICATIONS

PERIONS (CHAPERONS)

VIROIDS (only RNA) IN PLANTS

VIRUSES (DNA or RNA + CAPSID)

MYCOPLASMA & RICKETTSIAS & SPOROZOA

PROCARYOTES

EUKARYOTES

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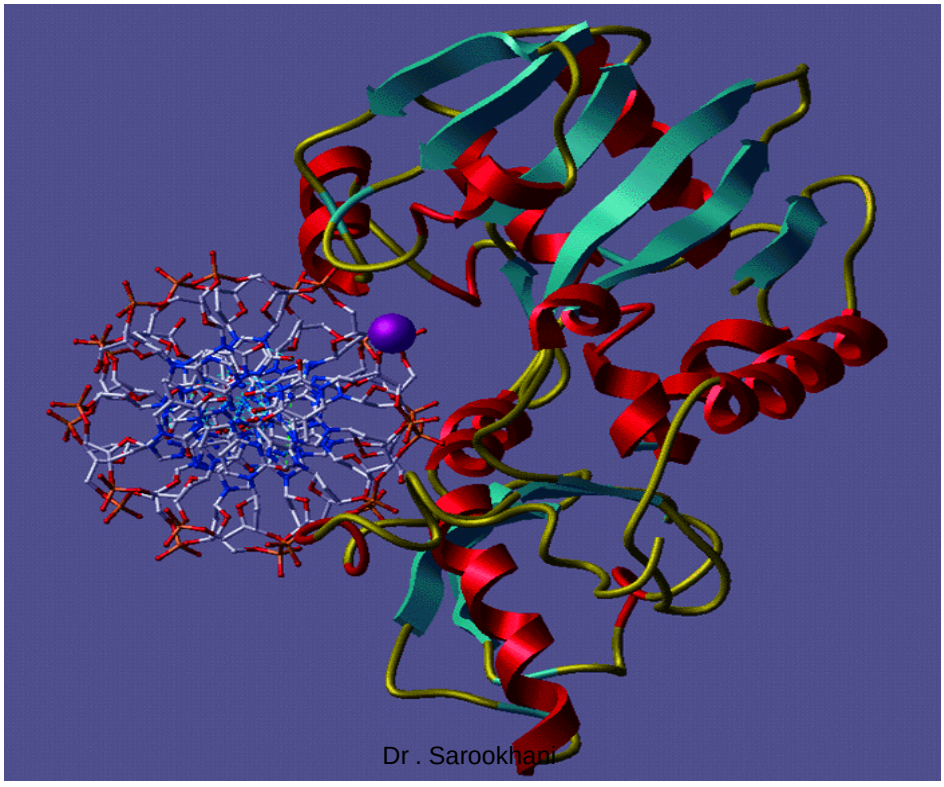
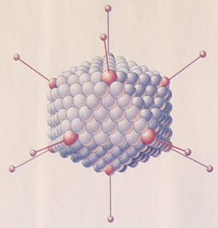
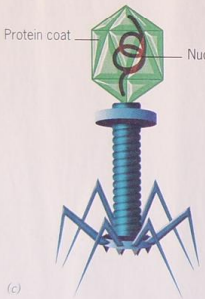
# Chaperon aided protein folding

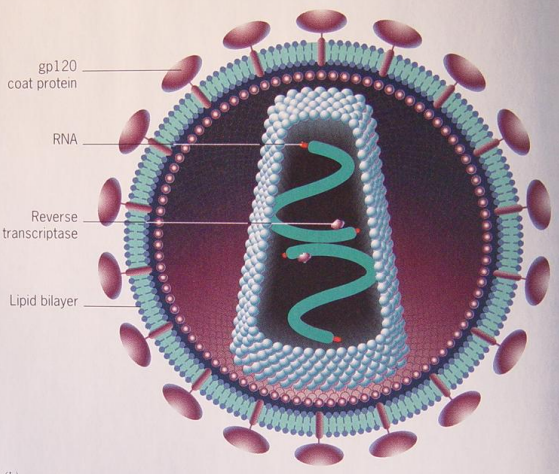




(a)




(c)




(b)

**FIGURE 1.21 Virus diversity.** The structures of (*a*) an adenovirus, (*b*) a human immunodeficiency virus (HIV), and (*c*) a T-even bacteriophage. Note: These viruses are not drawn to the same scale.

surrounded by a protein capsule, or *viroid*. Viri

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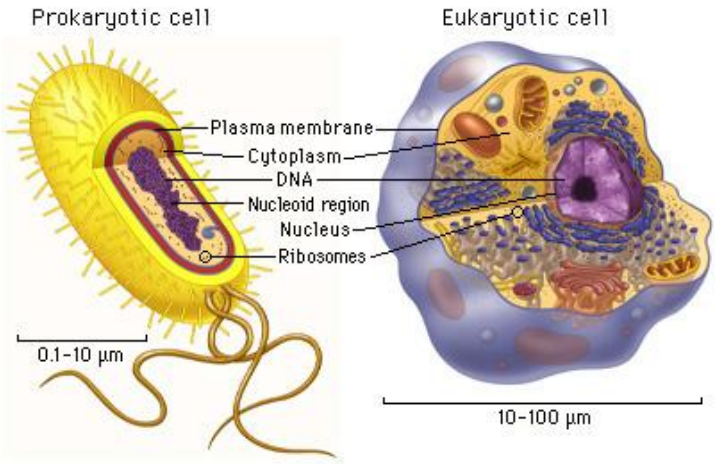
# Cell type classification

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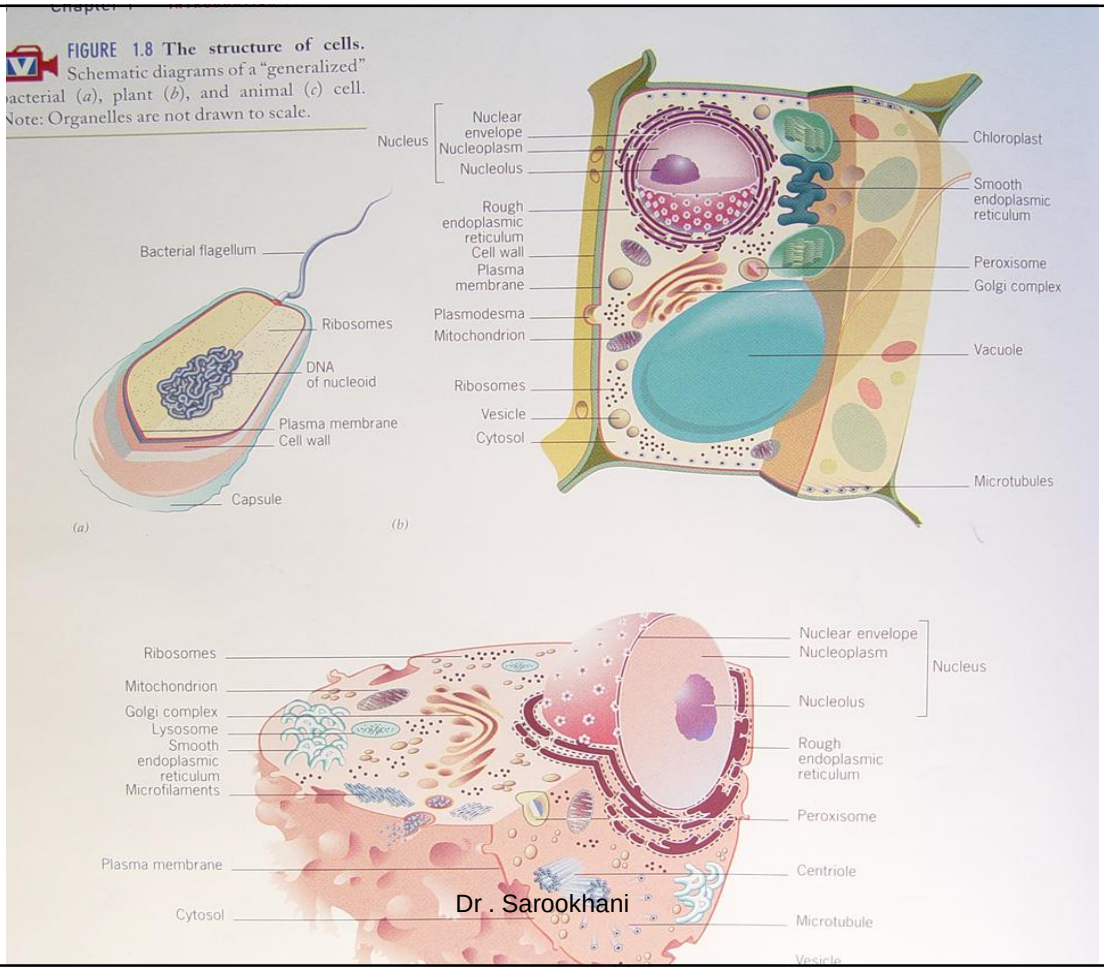


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# CELL STUDY

- Microscopic:
  the study of cell organizations and organels

- Physico-biochemical:
  the study of physico-biochemical reactions in the cell

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# MICROSCOPES

- Light microscope
- phase contrast microscope
- dark field microscope
- interference microscope
- polarizing microscope
- UV microscope(Immuno flourescence)
- electone microscope(EM)

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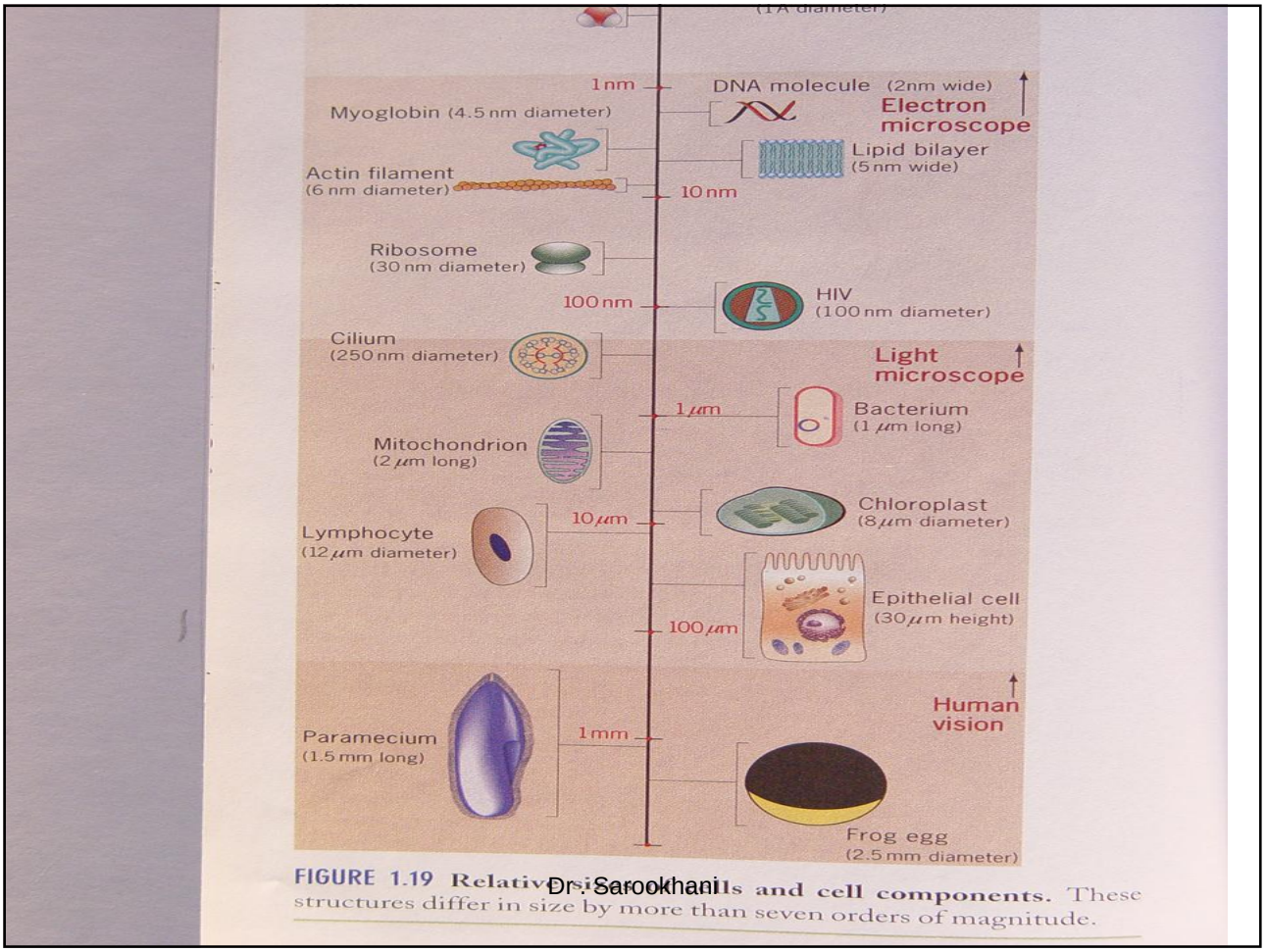


**FIGURE 1.19 Relative sizes of cells and cell components.** These structures differ in size by more than seven orders of magnitude.

# ELECTRONE MICROSCOPY

- transmission electron microscopy(TEM)

- Scanning electron microscopy (SEM)

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# TRANSMITANCE ELECTRONE MICROSCOPY


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# PHYSICO-BIOCHEMICAL TECHNIQUES FOR CELL STUDY

Cytochemistry
Auto radiography
ultra centrifugation
chromatography
electrophoresis
Spectro photometery
cell culture
Molecular methods

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# CYTOCHEMISTRY & HISTOCHEMISTRY

- Fe : prousian blue
- Lipids : Sudan black
- Nucleic acids : Folgen
- Proteins : Millon
- Polysaccharides : PAS
- Enzymes ( acid phosphatase, Dehydrogenases , Peroxidase )
- Immunocytochemistry ( F,R,Enz,Ferr)

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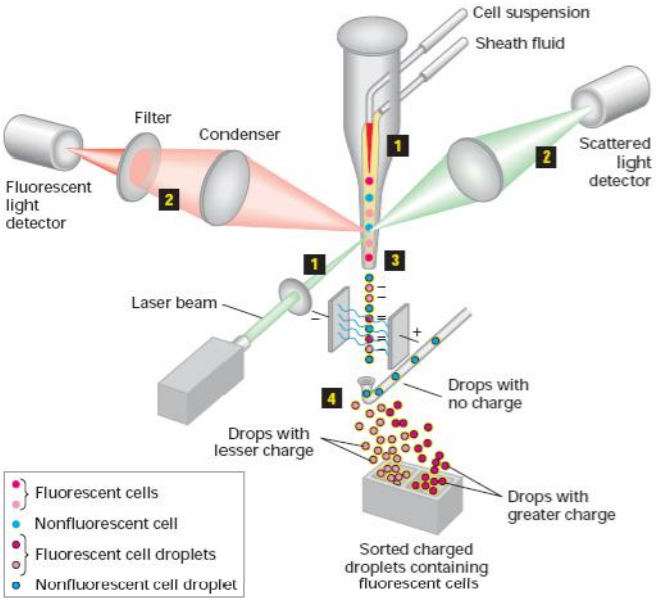


**◀ EXPERIMENTAL FIGURE 5-34**
**Fluorescence-activated cell sorter (FACS) separates cells that are labeled differentially with a fluorescent reagent.** Step 1: A concentrated suspension of labeled cells is mixed with a buffer (the sheath fluid) so that the cells pass single-file through a laser light beam. Step 2: Both the fluorescent light emitted and the light scattered by each cell are measured; from measurements of the scattered light, the size and shape of the cell can be determined. Step 3: The suspension is then forced through a nozzle, which forms tiny droplets containing at most a single cell. At the time of formation, each droplet is given a negative electric charge proportional to the amount of fluorescence of its cell. Step 4: Droplets with no charge and those with different electric charges are separated by an electric field and collected. It takes only milliseconds to sort each droplet, and so as many as 10 million cells per hour can pass through the machine. In this way, cells that have desired properties can be separated and then grown. [Adapted from D. R. Parks and L. A. Herzenberg, 1982, *Meth. Cell Biol.* **26**:283.]

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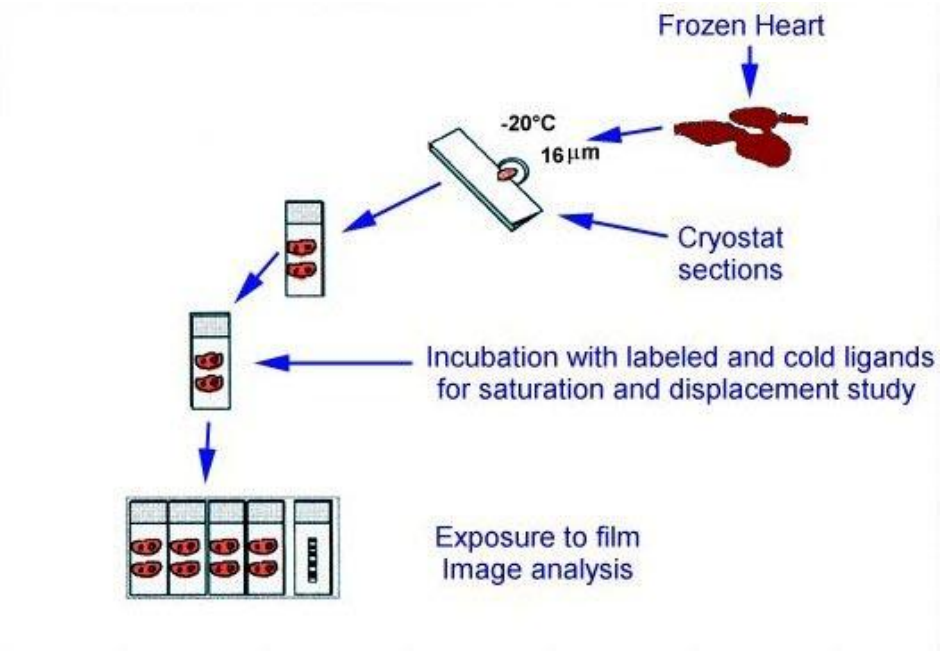
# AUTORADIOGRAPHY

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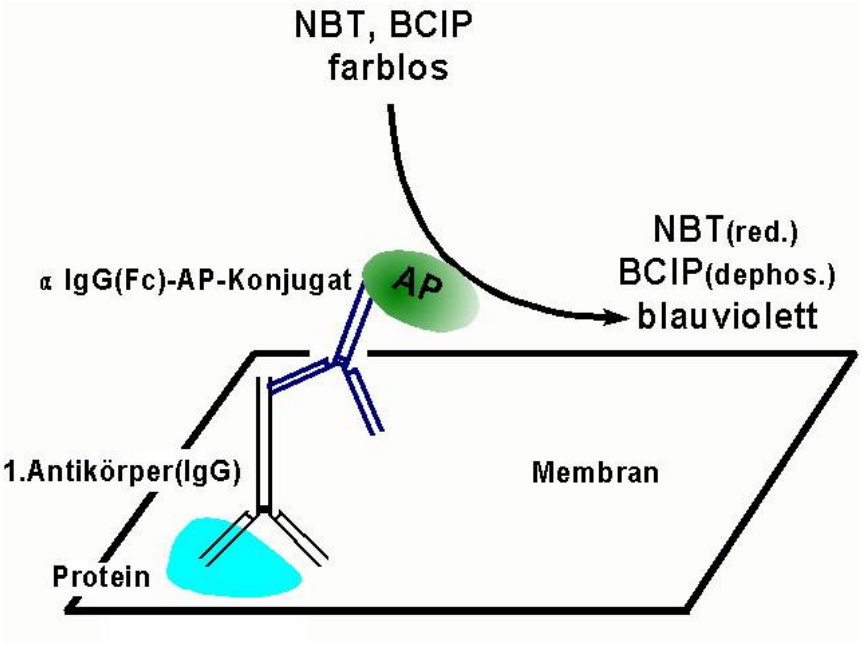
# Western blot

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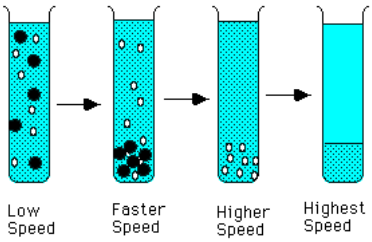
# ULTRA CENTRIFUGATION

Figure 2: Differential Centrifugation.

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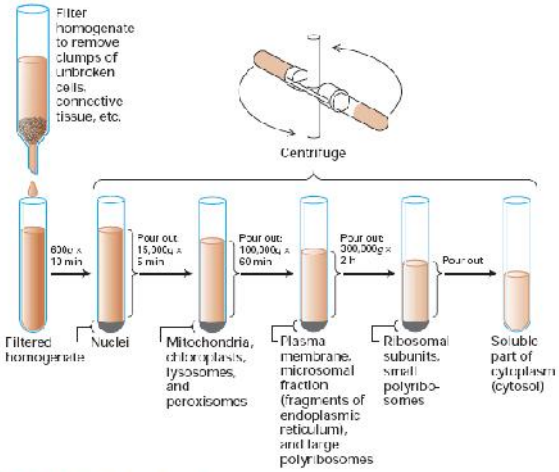


**▲ EXPERIMENTAL FIGURE 5-36 Differential centrifugation is a common first step in fractionating a cell homogenate.** The homogenate resulting from disrupting cells is usually filtered to remove unbroken cells and then centrifuged at a fairly low speed to selectively pellet the nucleus—the largest organelle. The undeposited material (the supernatant) is next centrifuged at a higher speed to sediment the mitochondria, chloroplasts, lysosomes, and peroxisomes. Subsequent centrifugation in the ultracentrifuge at 100,000*g* for 60 minutes results in deposition of the plasma membrane, fragments of the endoplasmic reticulum, and large polyribosomes. The recovery of ribosomal subunits, small polyribosomes, and particles such as complexes of enzymes requires additional centrifugation at still higher speeds. Only the cytosol—the soluble aqueous part of the cytoplasm—remains in the supernatant after centrifugation at 300,000*g* for 2 hours.

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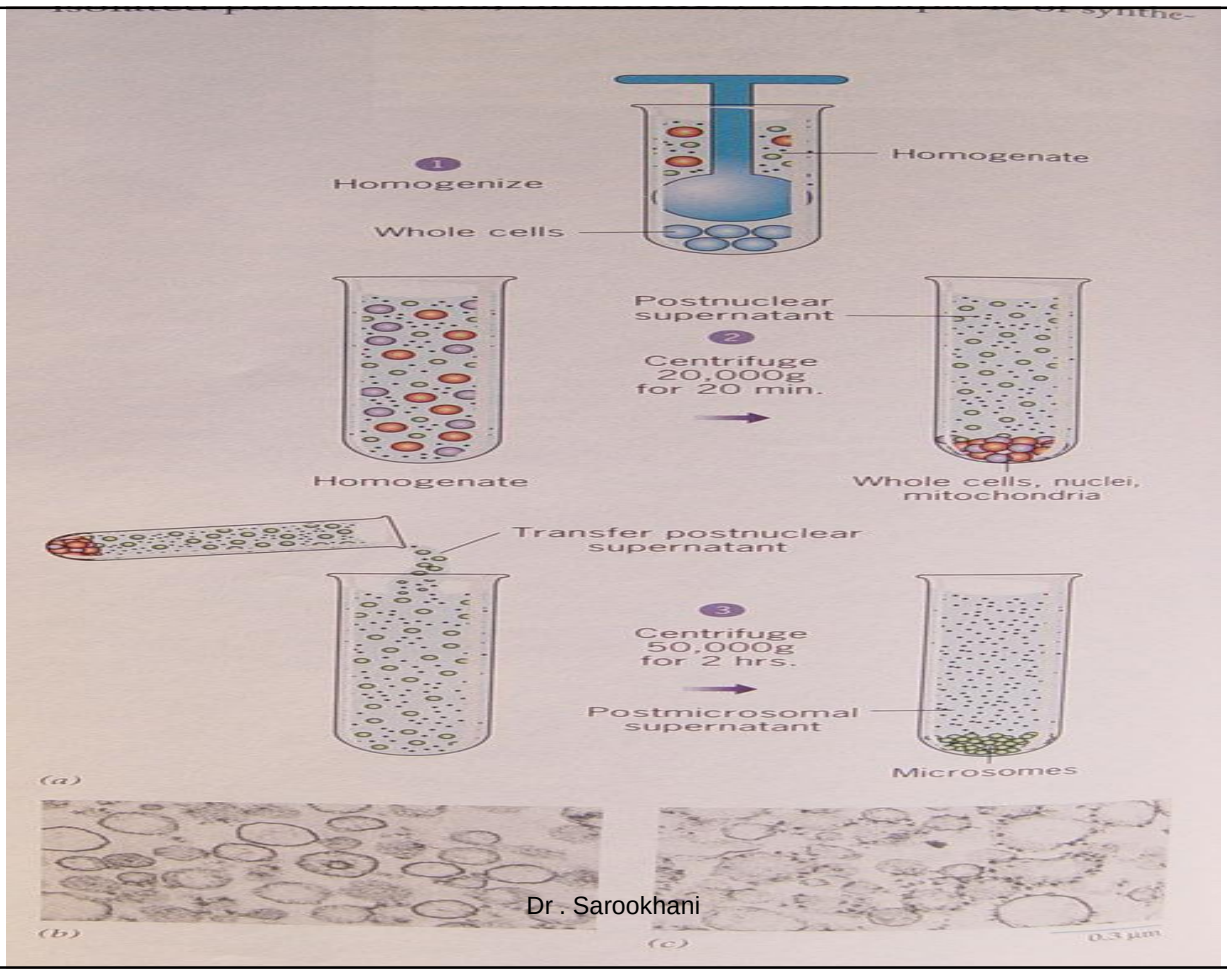


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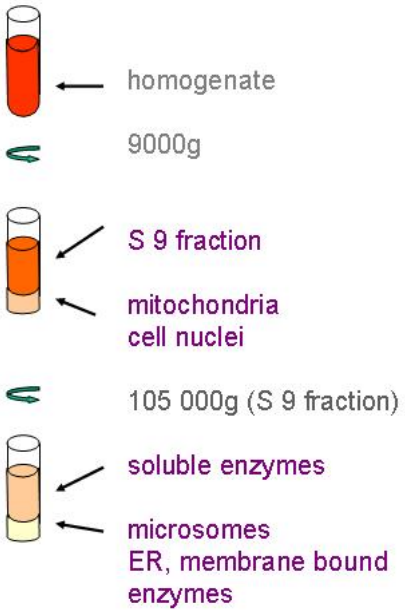
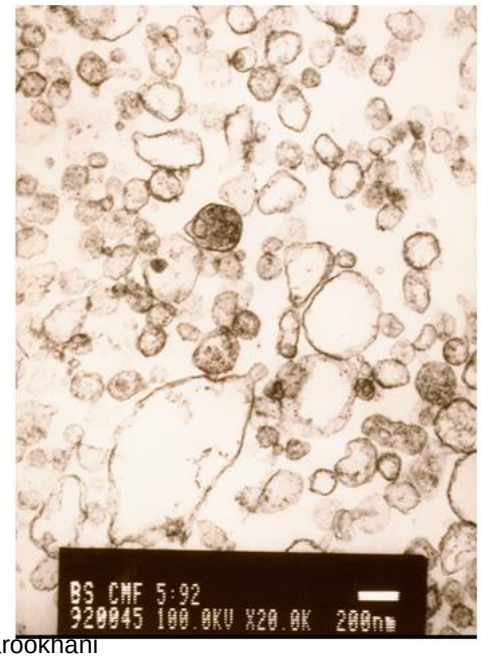
# CELL FRACTIONATION

(g=9.8 m/Cu s)

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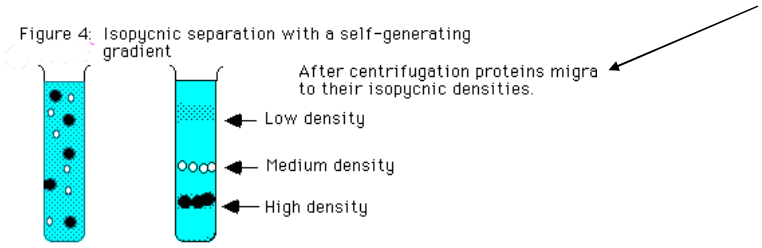
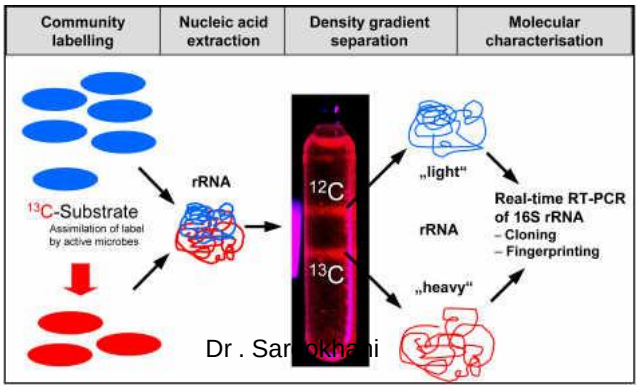
# ISOPYCNIC CENTRIFUGATION

(1S=17500 D)

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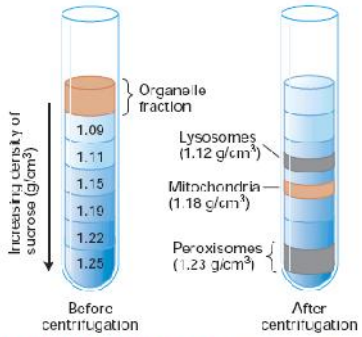


▲ **EXPERIMENTAL FIGURE 6-37 A mixed organelle fraction can be further separated by equilibrium density-gradient centrifugation.** In this example, material in the pellet from centrifugation at 15,000*g* (see Figure 6-36) is resuspended and layered on a gradient of increasingly more dense sucrose solutions in a centrifuge tube. During centrifugation for several hours, each organelle migrates to its appropriate equilibrium density and remains there. To obtain a good separation of lysosomes from mitochondria, the liver is perfused with a solution containing a small amount of detergent before the tissue is disrupted. During this perfusion period, detergent is taken into the cells by endocytosis and transferred to the lysosomes, making them less dense than they would normally be and permitting a "clean" separation of lysosomes from mitochondria

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Long Time:36-48 h

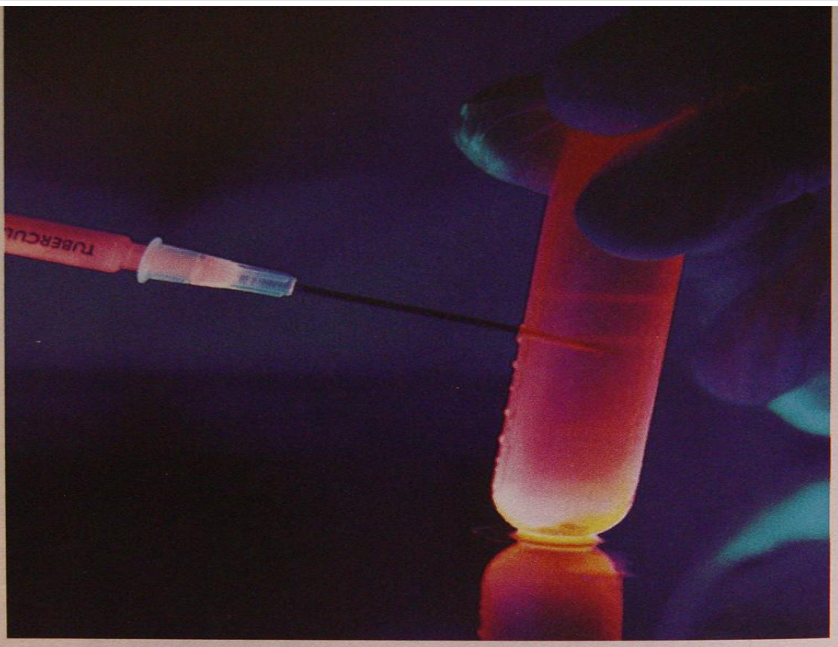
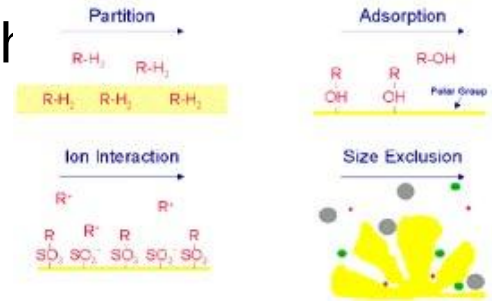


**FIGURE 18.41 Separation of plasmid DNA from that of the main bacterial chromosome by CsCl equilibrium centrifugation.** This centrifugation tube can be seen to contain two bands, one of plasmid DNA carrying a foreign DNA segment that has been cloned within the bacteria, and the other containing chromosomal DNA from these same bacteria. The two types of DNA have been separated during centrifugation (Figure 18.35*b*). The researcher is removing the DNA from the tube with a needle and syringe. The DNA in the tube is made visible using the DNA-binding compound ethidium bromide, which fluoresces under ultraviolet light. (PHOTOGRAPH BY TED SPEIGEL/CORBIS IMAGES.)

# CHROMATOGRAPHY

- absorption chromatography
- partition chromatography
- Gel filteration(size exclusion)
- Ion exchange chromatography
- Affinity chromatography
- Gas-liquid chromatograph
- HPLC

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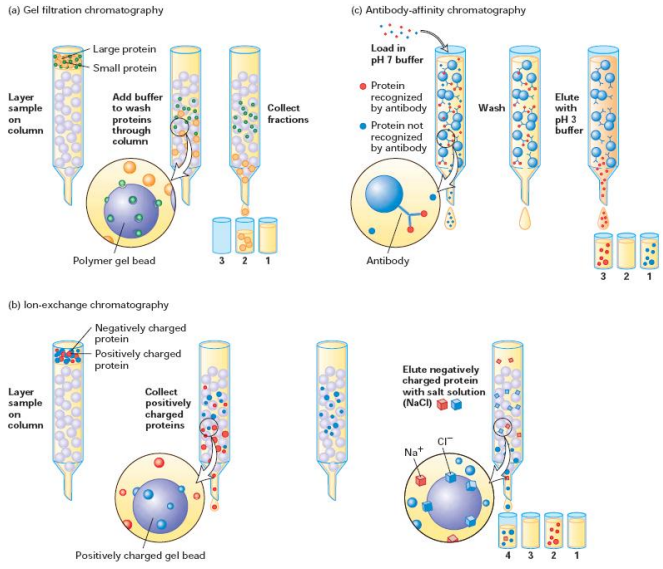


▲ **EXPERIMENTAL FIGURE 3-34 Three commonly used liquid chromatographic techniques separate proteins on the basis of mass, charge, or affinity for a specific ligand.** (a) Gel

having the same net charge as the beads are repelled and flow through the column, whereas proteins having the opposite charge bind to the beads. Bound proteins—in this case,

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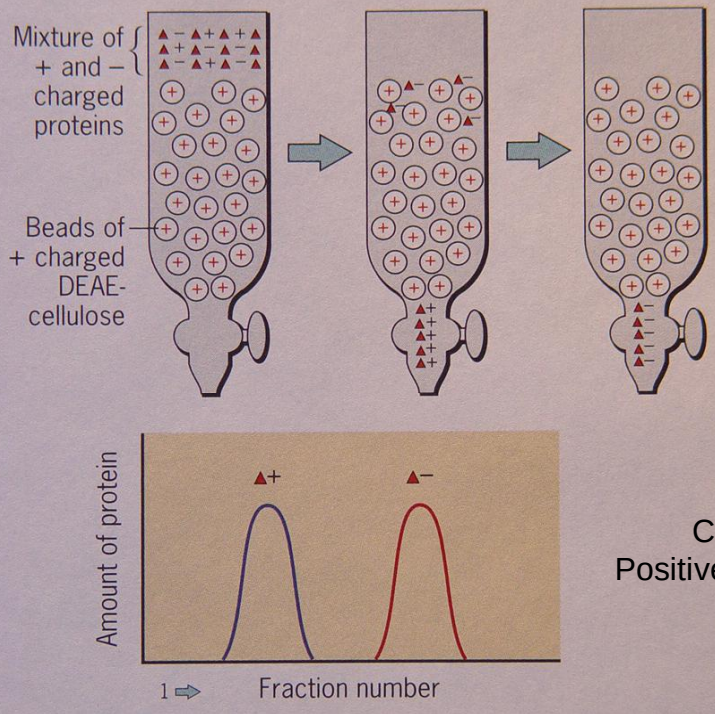


CM cellulose for
Positively charged proteins

**FIGURE 18.24 Ion-exchange chromatography.** The separation of two proteins by DEAE-cellulose. In this case, a positively charged ion-exchange resin is used to bind the negatively charged protein.

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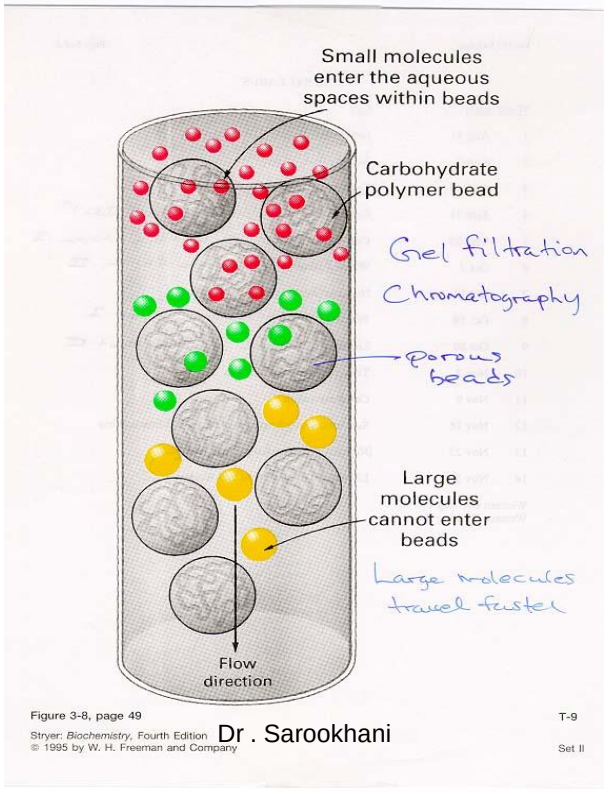
# GEL FILTERATION CHROMATOGRAPHY

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# AFFINITY CHROMATOGRAPHY

- SUBSTRATE / ENZYME
- LIGAND /BINDER
- Ag / Ab
- COMPLEMENTARY NUCLEOTIDE

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# High-Performance Liquid Chromatography (HPLC)

- **کروماتوگرافی مایع عملکرد عالی:**

**در کروماتوگرافی مایع، جداسازی بر اساس توزیع محلول بین ثابت(Constant Phase) و فاز متحرک (Liquid Phase)میباشد.**
**وقتی که در کروماتوگرافی مایع از یک ستون موثر و کارآمد استفاده میشود این تکنیک به عنوان کروماتوگرافی مایع با فشار بالا نیز نامیده میشود.**

**در آزمایشگاه بالینی از کروماتوگرافی مایع برای اندازه گیری داروها، ویتامین ها، هرمون ها، داروهای ضد آریتمی، ضد صرع، ضد درد، ضد افسردگی، پپتیدها و ویتامین ها استفاده می گردد.**

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# اجزاء دستگاه HPLC

- مخرن حلالHPLC
- پمپ یا پمپ ها
- تزریق کننده Injector
- آشکارسازDetector
- بی گاز کننده Degasser
- کامپیوتر

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# ستون Column

**ستون های آنالیتکال می توانند با مواد مختلفی پوشیده شوند بیشتر ستون ها از فلز ضد زنگ ساخته شده است. قطر داخلی یا ID آنها mm ۲-۵ و طول آنها mm۲۵۰-۱۰۰ متغیر است. متوسط قطر ذرات پوشاننده ۱۰-۲ میکرومتر می باشد که ستون با کیفیت بالا ایجاد می کند. پیش ستون ها Pre column و ستون های محافظ Guard Column برای افزایش طول عمر ستون‌های آنالیتکال بکار می رود.**

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۱) کروماتوگرافی فاز نرمال Normal Phase ch. که فاز متحرک محلول های غیر قطبی مانند تتراکلریدکربن، کلروفرم، بنزن و سیکلوهگزان می باشد. فاز ثابت دارای گروهای قطبی می باشد مانند سیانو(CN)، گروه آمینی($NH_2$) یا گروه دی اول(-CHOH-$CH_2OH$)

۲) کروماتوگرافی فاز معکوس Reveres phase ch. فاز متحرک قطبی می باشد که شامل متانول، آب، استونیتریل، محلول های بافری و فاز ثابت غیر قطبی است که متدوالترین آن کربن ۱۸ ($C_{18}$)و کربن ۸ ($C_8$)

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# آشکارساز Detector

**دتکتور HPLC برای شناسایی محلولی است که از ستون جدا میشود. این قسمت سیگنال های الکترونیکی متناسب با غلظت اجزاء نمونه را صادر می کند که به صورت پیک ثبت می شود.**

**انواع دکتور:**

۱. **دتکتور جذب UV**
۲. **دتکتور Fluorescence**
۳. **دتکتور Refractive Index**
۴. **دتکتور هدایتی( Conductivity)**
۵. **دتکتور Diode Array**

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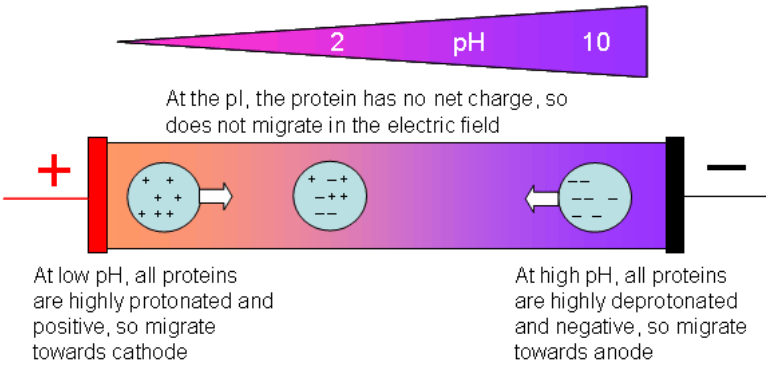
# ELECTROPHORESIS

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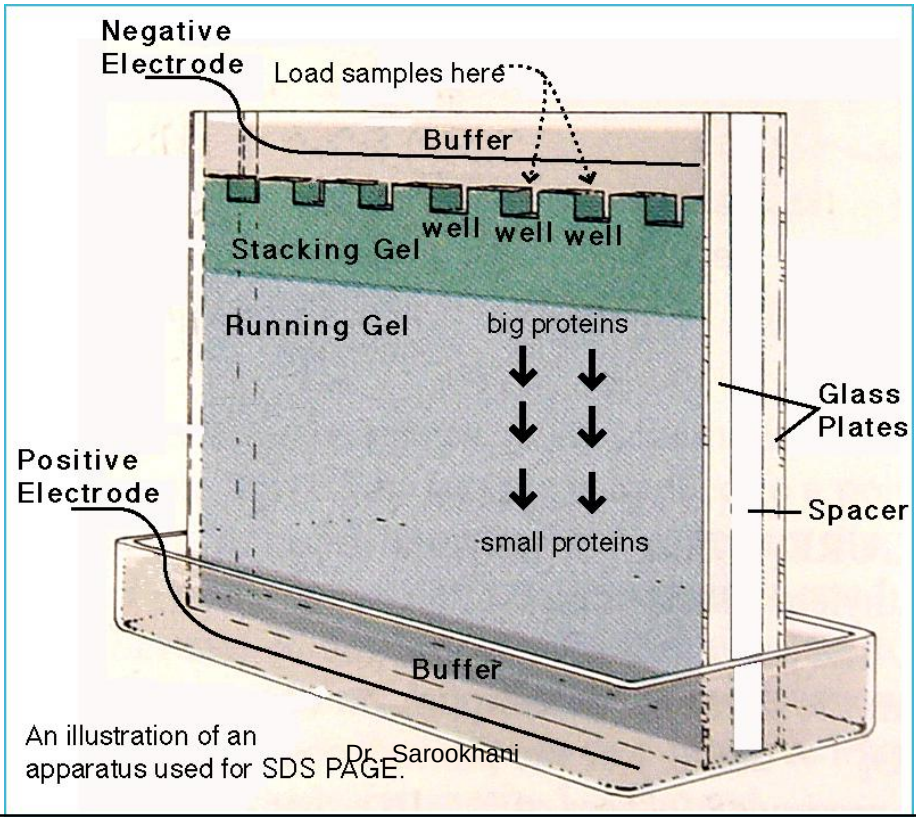
# SDS PAGE ELECTROPHORESIS




An illustration of an apparatus used for SDS PAGE. Dr. Sarookhani

# CELL CULTURE

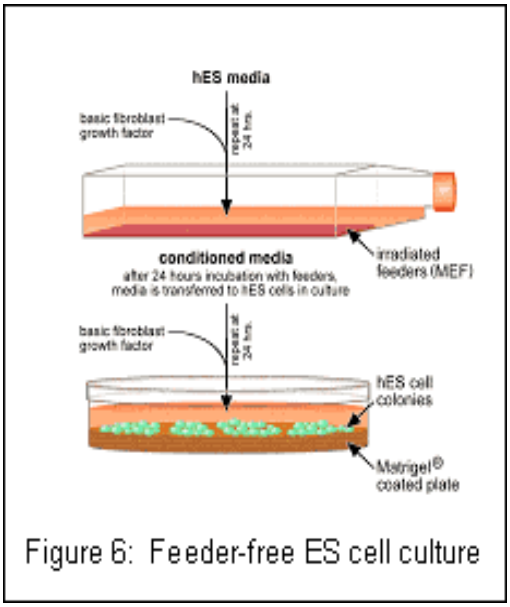



Figure 6: Feeder-free ES cell culture

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# MOLECULAR METHODS

- PCR & other amplification techniques
- nucleic acid hybridization techniques (blottings)
- use of  RE,s
- DNA sequencing analysis
- gene chip technology

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